Soil Microbial, Chemical and Physical Properties in Continuous Cotton and Integrated Crop–Livestock Systems

V. Acosta-Martínez,* T. M. Zobeck, and Vivien Allen

ABSTRACT

Continuous monoculture systems can reduce soil organic matter because of low organic inputs and disturbance from tillage practices. Integrated cotton (*Gossypium hirsutum*) cropping and livestock production systems in West Texas may provide more sustainable alternatives to the traditional continuous cotton system and improve soil quality. Our study was conducted on a Pullman soil (Fine, mixed, thermic Torrertic Paleustolls) after 5 yr as a complete randomized block design (three replications) that compared continuous cotton and an integrated livestock-crop system with a perennial warm-season grass pasture (*Bothriochloa bladhii*) paddock and two paddocks (two stages) of a rotation (wheat [*Triticum aestivum*]-fallow-rye [*Secale cereale*]-cotton). Total N (average: 1.0 g kg^{-1} soil) remained similar among systems and soil pH was >8.1. Organic C was higher (13.5 g kg^{-1} soil) in perennial pasture compared with continuous cotton (9.0 g kg^{-1} soil) at 0 to 5 cm. A similar trend was found for the soil aggregate stability. Soil microbial biomass C (C_{mic}) was greater in perennial pasture (193 mg kg^{-1} soil) and the rotation under rye and cotton (237 mg kg^{-1} soil) compared with continuous cotton (124 mg kg^{-1} soil) at 0 to 5 cm, and in perennial pasture at 5 to 10 and 10 to 15 cm. Soil microbial biomass N (N_{mic}) showed similar trends. Soil enzyme activities were greater in perennial pasture and the rotation (under rye and cotton) than under continuous cotton at 0 to 5 cm. The integrated crop-livestock system had higher protozoa (20:4ω6c = 1.98%) and fungi (18:3ω9c = 1.30%) than continuous cotton (20:4ω6c = 1.09%; 18:3ω9c = 0.76%). These findings suggest positive differences in soil function and sustainability of the integrated crop–livestock system compared with continuous cotton.

Conventional monoculture agricultural systems can reduce the quality of soils by loss of organic matter and structure because of low levels of organic inputs and regular disturbance from tillage practices. Cotton produced in the Texas High Plains region has been under continuous monoculture and conventional tillage systems that contribute to wind-induced soil erosion and reduce organic matter of these semiarid soils (V. Allen, unpublished data, 2004). Besides loss of quality in soils under continuous monoculture cotton systems, there is concern that irrigated cotton is using water from the Ogallala aquifer at rates that exceeds the water recharge. Thus, recent efforts are focused on developing integrated cotton cropping and livestock production systems that reduce water withdrawn from the Ogallala aquifer and that provide more conservative sustainable agricultural practices.

Crop rotations have positive effects on soil properties related to the higher C inputs and diversity of plant residues returned to soils in comparison with continuous systems (Miller and Dick, 1995; Entry et al., 1996; Friedel et al., 1996; Robinson et al., 1996; Moore et al., 2000). Conservation tillage practices (reduced or no-tillage) have shown to increase soil organic C (Franzluebbers et al., 1995b; Deng and Tabatabai 1996a, 1996b, 1997), enzyme activities (Deng and Tabatabai, 1996a, 1996b, 1997; Acosta-Martínez and Tabatabai, 2001; Acosta-Martínez et al., 2003), microbial biomass (Angers et al., 1993; Franzluebbers et al., 1994, 1995b) and to modify the soil microbial community (Frey et al., 1999; Pankhurst et al., 2002).

Soil quality assessments require an integration of physical, chemical, and microbiological measurements to reflect the diverse functions of soils (Doran and Parkin, 1994), but these properties may have different response times to soil management (Powlson et al., 1987; Kandeler et al., 1999). For example, microbial biomass C and N comprise only 1 to 3% of total soil C and up to 5% of total N in soils, respectively, but are the biologically the most active fraction of soil organic matter (Smith and Paul, 1990; Franzluebbers et al., 2001). The microbial biomass mediates many important functions in soils that include nutrient mineralization and cycling, and decomposition and formation of soil organic matter as they are the main source of enzymes in soils (Tabatabai, 1994). Enzymes are present in soil within various biotic and abiotic components (Burns, 1982).

The activities of enzymes, such as the hydrolases, can provide information on the status of key reactions that participate in rate limiting steps of the decomposition of organic matter and transformation of elements in soils. However, traditional assays determine only total soil enzyme activity and cannot distinguish the contribution from the different enzyme pools of soil, which may be affected differently by management. Klose and Tabatabai (1999a, 1999b, 2002) found that for arylsulfatase, chloroform fumigation of soil could be used to estimate intracytoplasmic enzyme activity vs. enzymes stabilized by soil organic matter and clay particles. Limitations of this method are the possible degradation of the released enzymes by soil proteases (Klose and Tabatabai, 1999a, 1999b, 2002; Renella et al., 2002) and/or their inactivation by chloroform (Klose and Tabatabai, 1999a). Furthermore, what is termed extracellular activity is likely coming from the periplasmic space, cell surface, and

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Abbreviations: C_{mic}, microbial biomass C; FAME, fatty acids methyl esters; N_{mic}, microbial biomass N; PCA, principal component analysis; PN, *p*-nitrophenol.

free enzymes stabilized (abionitic) in the soil matrix, and therefore does not distinguish total activity of microbial origin with activity from the abionitic form. None-theless, this method does provide additional information over total activity for how soil management affects cytoplasmic arylsulfatase activity, which would be expected to have some relationship with microbial biomass.

The objective of this work was to compare selected chemical, physical, and microbiological attributes under an integrated crop–livestock system and a conventional continuous cotton system that had been in place for 5 yr. The main focus of this study was on the integrated crop–livestock (ungrazed areas) and conventional continuous cotton systems as affected by their differences in crop–vegetation and tillage practices, without the confounding influence of livestock grazing.

MATERIALS AND METHODS

Experimental Design and Soil Management

Research initiated at the Texas Tech University field laboratory in 1997 compared continuous cotton and integrated crop–livestock systems. The farm is located in northeast Lubbock County in the Texas High Plains (101°47′ W longitude; 33°45′ N latitude; 993 m elevation). The landscape is characterized by nearly level soils with 0 to 1% slopes. The soil is a Pullman clay with 38% clay, 28% silt, and 34% sand. The research site is defined by a dry steppe climate with mild winters. Mean annual precipitation is 465 mm with most of the precipitation occurring from April through October.

The continuous cotton and integrated crop–livestock systems were replicated three times in a completely randomized block design with a total experimental land area of 12.75 ha. Each replication of the continuous cotton and integrated crop–livestock systems included 0.25 and 4 ha, respectively. Both systems were irrigated with an underground drip irrigation system with tapes located on 1-m centers and buried about 0.36 m deep in excess of potential evapotranspiration and variable volumes could be applied depending on crop water demands by adjusting duration of application. Soil test analyses indicated that no fertilizer other than N was needed. Chemicals, herbicides, and plant growth regulators were applied according to recommendations of integrated pest management specialists (V. Allen, unpublished data, 2004). A detailed description of the management of the systems has been previously provided (V. Allen, unpublished data, 2004), and thus, only a brief description will be provided here.

The continuous cotton system consisted of a single paddock. Soil was bedded on 1-m centers directly over drip irrigation tapes. 'Locket' wheat (*Triticum aestivum*) was planted each autumn in the furrow bottoms between the rows. Nitrogen (67 kg ha^{-1}) was applied through the irrigation system in late winter. In spring, wheat was chemically terminated with glyphosate [N-(phosphonomethyl) glycine, Monsanto Co, St. Louis, MO] (0.69 kg a.i. ha^{-1}), and 'Paymaster 2326RR' cotton was planted (193 750 seed ha^{-1}) into the top of beds in mid May each year.

The integrated crop–livestock system was composed of three paddocks. One paddock (2.1 ha), representing 53.5% of the system, was a perennial warm-season grass pasture of 'W.W.B. Dahl' old world bluestem (*Bothriochloa bladhii*) established in May 1997 (3.4 kg PLS ha^{-1}). Nitrogen (67 kg ha^{-1}) was applied in early August each year through the irrigation system. The remaining system (46.5%) land area was divided equally into two paddocks of 0.93 ha each. In these paddocks was a rotation of wheat, fallow, rye, and cotton at different stages such that both paddocks do not have the same crop at the same time. For example, beginning in 1997, rye was planted in early September, and was fertilized with 67 kg N ha^{-1} through the irrigation system. 'Maton' rye was terminated with glyphosate immediately before no-till planting of Paymaster 2326RR cotton (193 750 seed ha^{-1}) in May. Cotton was fertilized with N (67 kg ha^{-1}) through the irrigation system. Following cotton harvest in November, 'Locket' wheat was no-till planted into cotton stubble. Nitrogen (67 kg N ha^{-1}) was applied through the irrigation system in early spring. Wheat was terminated with glyphosate in June and the land was fallowed until rye was again planted the following September.

Although this study sampled the ungrazed areas of the integrated crop–livestock system (exclusion cages of 5.3 by 5.3 m), a brief description of the grazing sequence in the integrated crop–livestock system follows. The perennial pasture provided grazing intermittently for steers from January to mid-July, and a seed crop in October, but was sequence grazed with both rye and wheat as these forages were available for grazing. Cattle were moved from the perennial pasture to rye until early April and then to wheat until wheat was grazed out. While forages within the pasture were grazed, forages within excluded areas were managed as a hay crop and no grazing was allowed. Other than use as forage for hay instead of grazing, management within the excluded areas was similar to the entire paddocks of the integrated crop–livestock system, including fertilizer, irrigation, and time of planting.

Soil Sampling

Soil surface samples were taken in November 2002 and July 2003 at 0 to 5, 5 to 10, and 10 to 15 cm from the integrated crop–livestock and continuous cotton systems. One sample was taken from each field replication of the systems at each soil depth. Each sample was a composite mixture of 10 cores taken at random using a core sampler of 2.5-cm diam. The samples were taken from within areas (5.3 by 5.3 m) that were permanently excluded from grazing within the integrated crop–livestock system and from the whole area of continuous cotton system. The ungrazed areas of the integrated crop–livestock system were sampled to avoid including the additional factor and higher spatial variability created by livestock grazing activities in the comparison with continuous cotton system, which was never grazed. When crops were present, soil samples were combined from rhizosphere and non-rhizosphere areas. A separate soil sampling was conducted in July 2003 for determination of selected physical properties. A Giddings probe was used to collect samples at 0- to 5-, 5- to 10-, and 10- to 15-cm soil depths for determination of bulk density. Bulk soil samples were collected by shovel excavation from the same depths for determination of aggregate stability.

For convenience, the crop being produced will be in bold when November or July samplings are discussed independently. For example, in November the rye–cotton–**wheat**–fallow rotation was planted with wheat, wheat–fallow–**rye**–cotton rotation was under rye, and cotton was already harvested in the continuous cotton system. In July, the rye–cotton–wheat–**fallow** rotation was in fallow, wheat–fallow–rye–**cotton** rotation was in cotton and the continuous cotton system was under **cotton**.

Chemical and Physical Analyses

The pH values were measured in the air-dried soil (<2 mm) using a combination glass electrode (soil/water ratio 1:2.5). Organic C and total N were determined in air-dried soil (<180 μm) in a Vario Max-ELEMENTAR CN-analyzer (D-63452 Hanau,

Germany). Bulk density was determined by the core method (Blake and Hartge, 1986). Soil aggregate stability was determined on 2 g of soil (<1–2 mm air dried aggregates) by the method described by Kemper and Rosenau (1986). For this assessment, the soil was added to a 250-μm sieve, and was submerged into a crystal dish containing 50 to 80 mL of distilled water at a rate of 35 times min^{-1} for a total of 3 min ±5 s. The sieve was placed again into another crystal dish containing 50 to 80 mL of distilled water, sonicated for 30 s, and was submerged at a rate of 35 times min^{-1} for a total of 5 min to fully disrupt the stable aggregates and allow disintegration products to again collect in the dish. Material remaining on the screen includes coarse organic material and sand particles larger than 250 μm. The soils collected in the crystal dishes were dried in an oven at 110°C until the weight became constant to determine the stable aggregate fraction (%) according to the formula: [stable aggregates/(stable aggregates + unstable aggregates) × 100].

Microbial and Biochemical Analyses

The C_{mic} and N_{mic} were determined on a 15-g oven-dry equivalent field-moist soil sample (<2 mm) by the chloroform–fumigation–extraction method as described by Vance et al. (1987), using 0.5 *M* K_2SO_4 as an extractant. In brief, organic C and N from the fumigated (24 h) and non-fumigated (control) soil were quantified by a CN analyzer (Shimadzu Model TOC-V/$_{CPH}$–TN Shimadzu, Germany). The non-fumigated control values were subtracted from the fumigated values. The C_{mic} and N_{mic} were calculated using a k_{EC} factor of 0.45 (Wu et al., 1990) and k_{EN} factor of 0.54 (Jenkinson, 1988), respectively. Each sample had duplicate analyses and results are expressed on a moisture-free basis. Moisture was determined after drying at 105°C for 48 h.

The activities of β-glucosidase and β-glucosaminidase were assayed using 1 g of air-dried soil (<2 mm) with their appropriate substrate and incubated for 1 h (37°C) at their optimal pH as described in Tabatabai (1994) and Parham and Deng (2000), respectively. Arylsulfatase activity was determined in the field-moist soil (<2 mm) by the chloroform fumigation method of Klose and Tabatabai (1999a). Their method determines the arylsulfatase activity as described in Tabatabai (1994) in a set of samples fumigated with chloroform for 24 h in the absence of toluene, and on the non-fumigated counterparts. Arylsulfatase activity was estimated on the chloroform-fumigated samples, which is higher than non-fumigated counterparts due to a release of intracellular enzymes. Thus, intracellular activity (enzymes from microbial cell cytoplasm) was obtained by the difference of the activity of fumigated samples—activity of non-fumigated samples. The enzyme activities were assayed in duplicate with one control, to which substrate was added after incubation and subtracted from a sample value. The results were expressed in milligrams of *p*-nitrophenol (PN) released per kilogram of soil per hour.

Fatty acids were extracted from the soils using the procedure described for pure culture isolates by the Microbial Identification System (MIS, Microbial ID, Inc., Newark, DE) as previously applied for soil analyses (Cavigelli et al., 1995; Ibekwe and Kennedy, 1999; Acosta-Martínez et al., 2003). Briefly, the method consist of four steps: (1) saponification of fatty acids in 3 g of field-moist soil (<2 mm) with 3 mL of 3.75 *M* NaOH (methanol/water, 1:1) solution under heat (100°C) for 30 min; (2) methylation of fatty acids by adding 2 mL of 6 *M* HCl in aqueous methanol (1:0.85) under heat (80°C) for 10 min; (3) extraction of the fatty acid methyl esters (FAME) with 3 mL of 1:1 hexane/methyl-*tert* butyl-ether solution and rotating the samples end-over-end for 10 min, and (4) washing of the organic phases with 1.2% diluted NaOH by rotating the tubes end-over-end for 5 min. The organic phase (top phase) was analyzed in a 6890 GC Series II (Hewlett-Packard, Wilmington, DE) equipped with a flame ionization detector and 25 m by 0.2 mm fused silica capillary column using ultra high purity H as the carrier gas. The temperature program was ramped from 170 to 250°C at 5°C min^{-1}. Fatty acids were identified and their relative peak areas (percentage) were determined with respect to the other fatty acids in a sample using the MIS Aerobe method of the MIDI system (Microbial ID, Inc., Newark, DE). The FAMEs are described by the number of C, followed by a colon, the number of double bonds and then by the position of the first double bond from the methyl (ω) end of molecules, and *cis* and *trans* isomers are indicated by c or t, respectively. Branched fatty acids are indicated by the prefixes *i* and *a* for iso and anteiso, respectively.

Statistical Analyses

The data were analyzed as a completely randomized block design model. For the chemical, physical, and most microbiological properties, analysis of variance (ANOVA) and separation of means by least significant differences (LSD) at $P < 0.05$ were performed by using the general linear model procedure (SAS Institute, 1999). In the FAME analysis, principal component analysis (PCA) was used to demonstrate the similarities and differences in the FAME profiles among samples from integrated crop–livestock and continuous cotton systems by including all the fatty acids extracted. A contrast comparison was performed for the fatty acids extracted from soils that have been suggested as fungi, bacteria, or protozoa indicators (Bossio et al., 1998; Zelles, 1999). The contrast comparison was non-orthogonal to solve the following hypotheses: (1) continuous cotton system will differ from perennial pasture; (2) continuous cotton system will differ from rye–cotton–wheat–fallow rotation; and (3) continuous cotton system will differ from wheat–fallow–rye–cotton rotation.

RESULTS

Chemical and Physical Properties

The ANOVA revealed that soil pH, organic C, and total N were not affected by sampling time, but were affected by soil depth ($P < 0.001$) (Table 1). Soil pH was higher only in continuous cotton system in comparison with integrated crop–livestock system at the 0- to 5-cm depth in November 2002, but it was similar among these systems in July 2003. Total N did not show differences among systems. In both samplings, soil organic C content was only higher at the 0- to 5-cm depth in perennial pasture compared with continuous cotton. In July at the 0- to 5-cm depth, the aggregate stability and bulk density were significantly higher in perennial pasture than in continuous cotton (Table 1).

Microbial Biomass Carbon and Nitrogen

The soil C_{mic} and N_{mic} were higher in November 2002 than in July 2003 ($P < 0.001$), and showed this trend ($P < 0.05$) with soil depth: 0- to 5- > 5- to 10- > 10- to 15-cm depths (Fig. 1). In both samplings, soil C_{mic} was greater in perennial pasture and wheat–fallow–**rye**–cotton rotation compared with continuous cotton at the 0- to 5-cm depth (Fig. 1A, 1B). In November, soil C_{mic} was

Table 1. Soil chemical and physical properties after 5 yr in the integrated crop–livestock and continuous cotton systems.

	Integrated crop–livestock system†						Continuous cotton system	
	Perennial pasture		Wheat–fallow–(rye–cotton)		Rye–cotton–(wheat–fallow)		Continuous cotton	
Depth	Nov-02	Jul-03	Nov-02 (rye)	Jul-03 (cotton)	Nov-02 (wheat)	Jul-03 (fallow)	Nov-02	Jul-03
cm								
				Soil pH (H_2O)				
0–5	8.2ab‡	8.2a	8.2ab	8.1a	8.1b	8.2a	8.4a	8.3a
5–10	8.4a	8.4a	8.5a	8.3a	8.4a	8.3a	8.4a	8.4a
10–15	8.5a	8.5a	8.1a	8.3b	8.3a	8.4ab	8.3a	8.4a
				Organic C, g kg^{-1} soil				
0–5	13.3a	13.6a	11.2ab	10.6b	10.7ab	11.5ab	8.5b	9.5b
5–10	9.6a	9.5a	8.7ab	8.7a	8.3b	8.9a	8.3b	8.4a
10–15	8.0a	8.3a	8.4a	7.8a	6.7a	7.5a	8.5a	7.5a
				Total N, g kg^{-1} soil				
0–5	0.9a	1.0a	1.0a	1.0a	1.1a	1.1a	0.9a	0.8a
5–10	0.7a	0.8a	0.8a	0.9a	0.8a	0.9a	0.7a	0.8a
10–15	0.7a	0.7a	0.7a	0.7a	0.8a	0.7a	0.7a	0.7a
				Aggregate stability, %				
0–5	–	39a	–	37ab	–	–	–	21b
5–10	–	23a	–	22a	–	–	–	24a
10–15	–	25a	–	27a	–	–	–	22b
				Bulk density, Mg m^{-3}				
0–5	–	1.4a	–	1.3a	–	–	–	1.1b
5–10	–	1.4a	–	1.4a	–	–	–	1.3b
10–15	–	1.5a	–	1.5a	–	–	–	1.4b

† In the Integrated crop–livestock system, the rotation was sampled under rye or wheat in November and under cotton or fallow in July.
‡ A similar letter at the same depth and time of sampling indicates no significant differences between treatments according to LSD ($P < 0.05$).

greater in perennial pasture and wheat–fallow–**rye**–cotton rotation compared with continuous cotton at the 5- to 10-cm depth (Fig. 1A). In both samplings, soil C_{mic} was greater in the integrated crop–livestock system compared with continuous cotton system at the 10- to 15-cm depth.

In November, soil N_{mic} showed the following trend at the 0- to 5-cm depth: perennial pasture = wheat–fallow–**rye**–cotton rotation > rye–cotton–**wheat**–fallow rotation > continuous cotton (Fig. 1C). Perennial pasture and wheat–fallow–**rye**–cotton rotation revealed greater N_{mic} than in continuous cotton at the 5- to 10-cm depth. Higher N_{mic} was found in the integrated crop–livestock system than in continuous cotton at 10 to 15 cm. In July, soil N_{mic} was greater in perennial pasture and wheat–fallow–rye–**cotton** rotation at the 0- to 5-cm depth compared with continuous cotton, and greater in perennial pasture and wheat–fallow–rye–**cotton** rotation compared with continuous cotton system at the 5- to 10- and 10- to 15-cm depths (Fig. 1D).

The C_{mic}/N_{mic} ratio did not show the trends of the microbial biomass. This ratio was higher only in perennial pasture compared with continuous cotton in July. On average at 0- to 5-cm depth, C_{mic}/N_{mic} ratio was 8.2 and 7.9 in November 2002, and July 2003, respectively.

Enzyme Activities

The enzyme activities were higher in November 2002 than in July 2003 ($P < 0.01$), and always higher at 0 to 5 cm than the lower soil depths ($P < 0.001$) (Fig. 2). β-glucosidase activity was generally greater in perennial pasture and wheat–fallow–**rye**–cotton rotation compared with continuous cotton at the 0- to 5-cm depth (Fig. 2A, 2B). There were generally no differences among systems at the 5- to 10-cm depth, and only greater enzyme activities were found in rye–cotton–**wheat**–fallow compared with continuous cotton at the 10- to 15-cm depth in November.

In November, β-glucosaminidase activity at the 0- to 5-cm soil depth showed this significant ($P < 0.05$) trend among systems: pasture > wheat–fallow–**rye**–cotton > rye–cotton–**wheat**–fallow > continuous cotton (Fig. 2C). The activity of this enzyme was greater in perennial pasture in comparison with continuous cotton at the 5- to 10-cm depth, and there were no differences among systems at the 10- to 15-cm depth. In July, the activity of this enzyme was greatest in perennial pasture at the 0- to 5- and the 5- to 10-cm depths (Fig. 2D).

The ANOVA revealed that the arylsulfatase activity of fumigated and non-fumigated soil was affected by sampling time and soil depth ($P < 0.001$). Arylsulfatase activity was greater in the integrated crop–livestock system compared with continuous cotton system at the 0- to 5-cm depth (Fig. 3). Arylsulfatase activity of the non-fumigated soil represented only 22% (November 2002) to 34% (July 2003) of the total activity of the fumigated soil.

FAME Profiles

Principal component analyses of all fatty acids extracted (up to 120 fatty acids) from the soils showed that the microbial community structure of perennial pasture differed from that of rye–cotton–wheat–fallow, wheat–fallow–rye–cotton, and continuous cotton for all depths along PC2 in November (PC2 = 19%) and July (PC2 = 18%) (Fig. 4). The PCA accounted for 71 and 66% of the variability among systems in November 2002 and July 2003, respectively.

The ANOVA showed that the microbial FAMEs

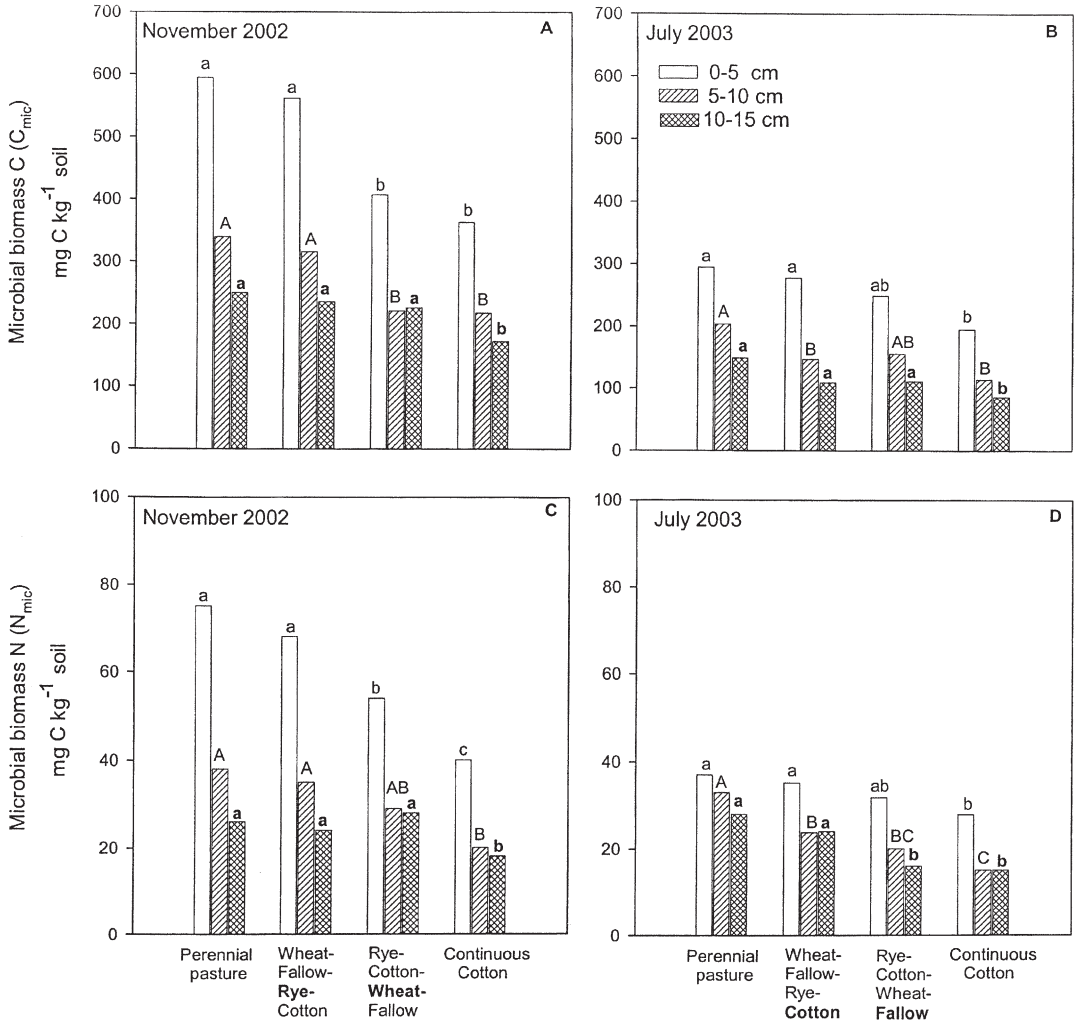

Fig. 1. (A, B) Microbial biomass C and (C, D) N in soils under integrated crop–livestock system and continuous cotton system in November 2002 and July 2003. The bold crop signifies which crop the field is under at the time of the measurements. Similar letter at the same depth indicates no significant treatment differences according to least significant differences (LSD) at $P < 0.05$.

abundance were affected by the treatments ($P < 0.05$), but were not affected by sampling time and soil depth. Thus, Table 2 provides an average of microbial FAMEs abundance from both sampling times and the three soil depths evaluated (0–5, 5–10, and 10–15 cm). Contrast comparison showed greater 20:4ω6c levels in the integrated crop–livestock system in comparison with continuous cotton. The contrast comparisons for fungi and bacteria FAMEs showed that not all fatty acids within a group had the same trends among systems. Among fungi FAMEs, compared with continuous cotton, there was greater ($P < 0.001$) 18:3ω6c in the integrated crop-livestock system, whereas 18:2ω6c and 18:1ω9c showed only greater ($P < 0.01$) abundance in perennial pasture. Among the bacteria FAMEs, 18:1ω7c showed greater abundance in perennial pasture ($P < 0.001$) in comparison with continuous cotton. There was greater abundance of 15:0 in perennial pasture ($P < 0.001$) and wheat–fallow–rye–cotton ($P < 0.01$) compared with continuous cotton. The other bacteria indicators (*i*15:0, a15:0, and *i*16:0) showed generally similar abundance in perennial pasture and continuous cotton.

In this soil, there was higher abundance of fungi (18:2ω6c or 18:1ω9c) compared with bacteria (*i*15:0, *a*15:0, 15:0, or *i*16:0) indicators.

DISCUSSION

The bulk density measured in July 2003 suggested a higher level of compaction in soils under integrated crop–livestock system due to the effects of no-tillage in comparison with the continuous cotton system at the end of 5 yr. The higher ($P < 0.05$) aggregate stability in perennial pasture (0- to 5-cm depth) indicates changes on organic matter quantity and quality have occurred in soil (Lynch and Bragg, 1985). The changes found on the physical parameters may have been due to the surface cover, rhizosphere effects, crop rotations, and lack of tillage practices in the integrated crop–livestock system.

In November 2002, the integrated crop–livestock system had winter cover crops in the rotation or permanent vegetation in the pasture, whereas the continuous cotton system was already harvested. Thus, samples were taken again in the 2003 summer growing season to allow a

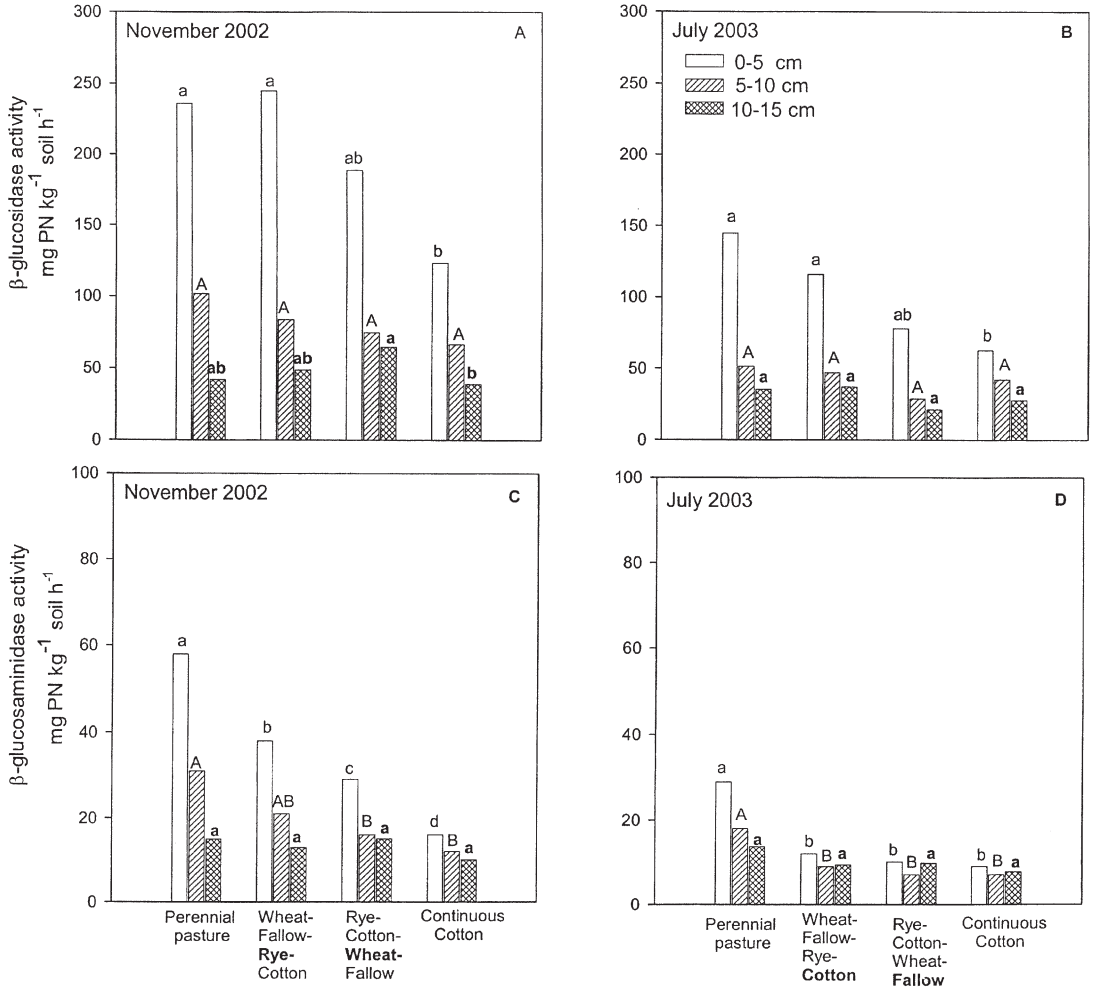

Fig. 2. (A, B) β-glucosidase and (C, D) β-glucosaminidase activities in soils under integrated crop–livestock system and continuous cotton system in November 2002 and July 2003. The bold crop signifies which crop the field is under at the time of the measurements. Similar letter at the same depth indicates no significant treatment differences according to least significant differences (LSD) at $P < 0.05$.

better evaluation of management-history trends among systems. The lower C_{mic}, N_{mic}, C_{mic}/N_{mic}, and the enzyme activities in July 2003 occurred as a consequence of higher temperatures and lower precipitation, and thus, lower soil moisture than in November 2002 (V. Allen, unpublished data, 2004). The similar soil pH, organic C, and total N in the two samplings are in agreement with previous findings that microbiological properties such as enzyme activities, respiration, and C_{mic} responded more quickly to environmental conditions than organic matter (Brookes, 1995).

Organic C was not affected by which crop was sampled in the rotation, but microbiological and biochemical parameters were affected by crop type. When the rotation was sampled under rye or cotton (wheat–fallow–**rye–cotton**), there was always a significant ($P < 0.05$) effect on soil microbial and biochemical properties compared with continuous cotton, but this was not always true for wheat or fallow (rye–cotton–**wheat–fallow**). This indicates rye had greater impacts than wheat (November) or cotton than fallow (July) on soil rhizosphere microbial populations. It may also demonstrate the significant increases that occur on microbial populations and activities of soil by growing crops after fallow periods (wheat–fallow–**rye–cotton**).

The microbial properties, C_{mic}, N_{mic}, and β-glucosaminidase activity were more sensitive for detecting differences in soil management than organic matter properties (total C and N). The microbiological properties often showed depth effects whereas total N remained similar among systems and organic C was only significantly greater at the 0- to 5-cm depth between perennial pasture and continuous cotton. These findings are in agreement with previous work that reported the microbial biomass, enzyme activities, and respiration responded more quickly to crop management practices than organic matter (Powlson et al., 1987; Brookes, 1995). Similarly, 7 yr of winter cover cropping showed no effect on organic C levels, but microbial biomass and β-glucosidase activity (0- to 7.5-cm depth) were positively affected (Ndiaye et al., 2000). Other studies using different crops that varied in amount, rate of decomposition and quality of residue inputs showed effects on soil microbial and biochemical properties (Janzen and Lucey 1988; Franzluebbers et al., 1995a, 1995b; Klose et al., 1999; Ekenler and Tabatabai, 2002). The sensitivity of microbial properties is likely due to much faster turnover of microbial biomass than soil organic matter (Sparling, 1997).

The measurement of enzyme activities indicated the

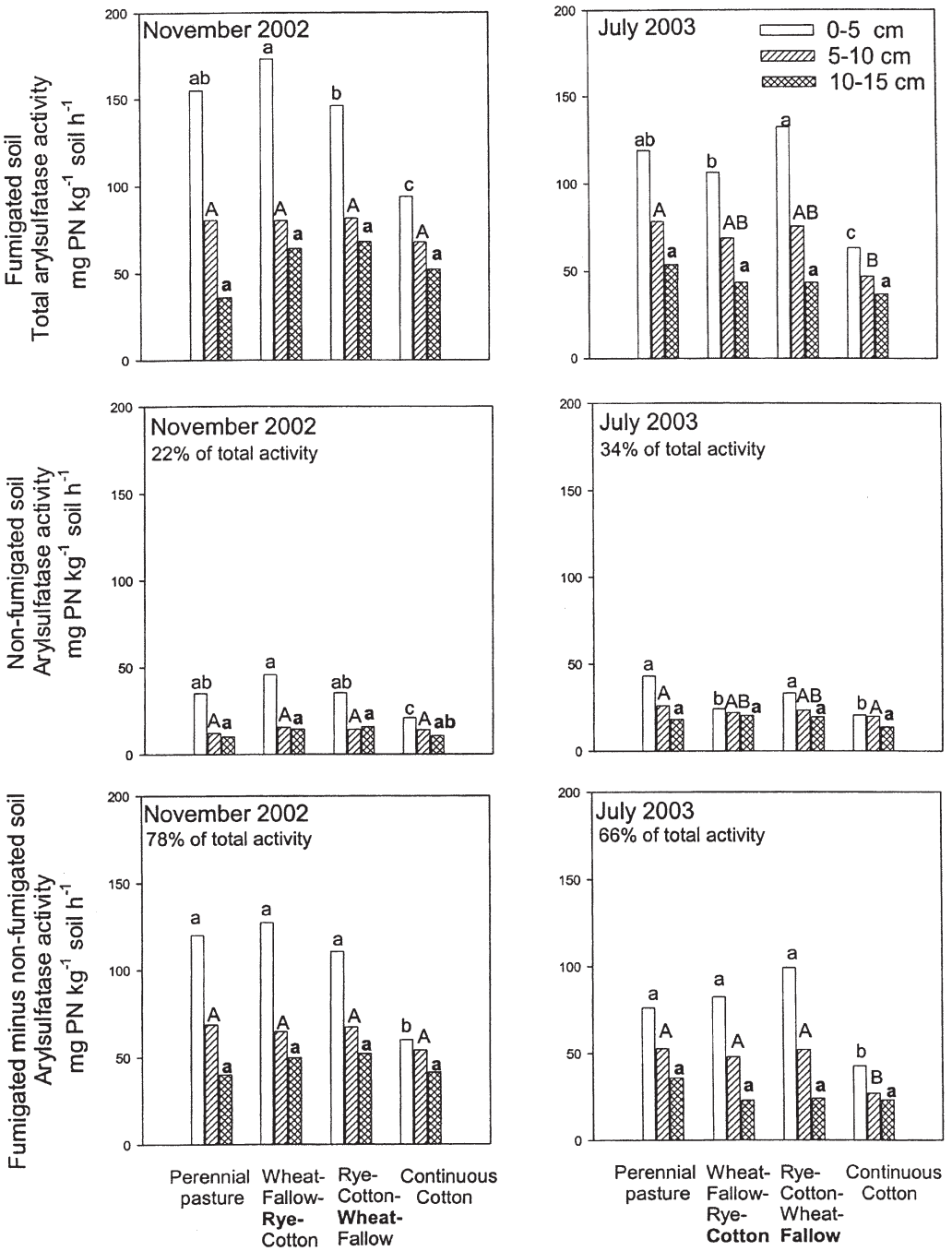

Fig. 3. Arylsulfatase activity of chloroform fumigated soil, non-fumigated, and fumigated minus non-fumigated soil of the integrated crop-livestock system and continuous cotton system in November 2002 and July 2003. The bold crop signifies which crop the field is under at the time of the measurements. Similar letter at the same depth indicates no significant treatment differences according to least significant differences (LSD) at $P < 0.05$.

sensitivity of reactions involved in the degradation of cellulose (β-glucosidase) or chitin (β-glucosaminidase) and organic S (arylsulfatase) compounds transformations in soils to the different cropping systems and vegetation studied. Ndiaye et al. (2000) found that microbial biomass, and the activities of arylsulfatase and β-glucosidase were sensitive to alternative management practices after only 1 to 2 yr of winter cover plots at 0- to 7.5-cm depth. In this study, β-glucosaminidase activity was more sensitive than the other enzymes as it showed differentiation of perennial pasture and continuous cotton at the 5- to 10-cm depth. The changes of β-glucosaminidase activity under the integrated crop–livestock system have important implications for the soil N cycling dynamics because this enzyme activity has been significantly correlated with N mineralization (Ekenler and Tabatabai, 2002).

The fact that soil C_{mic}, N_{mic}, and enzyme activities responded similarly to management may lead to the assumption that the source and location of enzymes in soils is more related to the microbial biomass (Bandick and Dick, 1999; Ndiaye et al., 2000). This study esti-

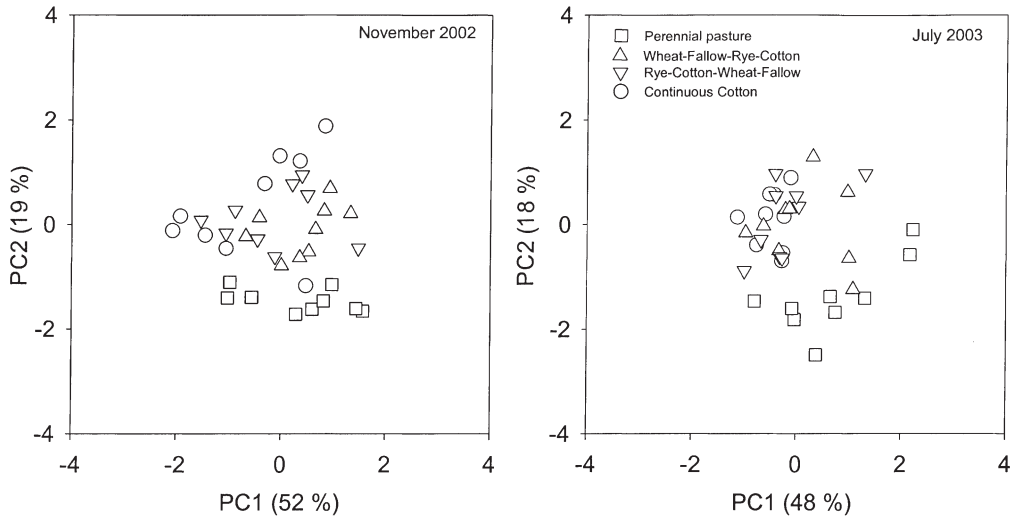

Fig. 4. Principal component analyses (PCA) of fatty acid methyl ester (FAME) profiles of soils under integrated crop–livestock system and continuous cotton system in November 2002 and July 2003.

mated only arylsulfatase activity from the microbial biomass with the chloroform fumigation method of Klose and Tabatabai (1999a) because the method does not work for the other enzymes investigated (Klose and Tabatabai, 2002). Renella et al. (2002) suggested the requirement of a complete inhibition of active proteases to determine the intracellular activity with the chloroform method (Klose and Tabatabai, 1999a). In addition, non-fumigated soil may not represent only the extracellular activity of the enzyme stabilized in the soil matrix (Klose and Tabatabai, 1999a; Renella et al., 2002). Taking into consideration these limitations, total arylsulfatase activity can be estimated on the chloroform-fumigated soil because fumigation causes the release of intracellular enzymes from the microbial cell cytoplasm that may not be determined in the non-fumigated soil without toluene. Thus, the results demonstrated the sensitivity of arylsulfatase activity, including the intracellular enzymes of the microbial cell cytoplasm, to soil management (i.e., crop types, vegetation, and tillage). However, since fumigation is needed to expose the intracellular arylsulfatase to the substrate, it is unlikely that this intracellular enzyme is directly involved in the mineralization of organic S compounds of soil as it appears they could not pass the cytoplasmic membrane (R.P. Dick, personal communication, 2004).

Among methods to study microbial biomass composition in soils, FAME analyses have become an important tool to detect changes in the relative abundance of FAME profiles and of indicator FAMEs for fungi (18:2ω6c, 18:3ω6c, 18:1ω9c), bacteria (18:1ω7c, *i*15:0, *a*15:0, 15:0 and *i*16:0), or protozoa (20:4ω6c) as affected by management (Klug and Tiedje, 1993; Bossio et al., 1998; Ibekwe and Kennedy, 1999; Schutter et al., 2001). The FAME analysis can present an advantage to culturing methods because it avoids selectivity toward fast-growing microorganisms in the media, but one limitation of this analysis is that some fatty acids may not come from living organisms but rather from the soil matrix. Furthermore, fatty acids used as biomarkers may be also present in

Table 2. Microbial indicator fatty acids after 5 yr in the integrated crop–livestock and continuous cotton systems.

	Integrated crop–livestock system§						Continuous cotton system
FAME indicator†	Perennial pasture	Contrast‡	Wheat–fallow–(rye–cotton)	Contrast	Rye–cotton–(wheat–fallow)	Contrast	Continuous cotton
	%		%		%		%
Protozoa							
20:4ω6c	2.30¶	0.0004	1.92	0.003	1.72	0.008	1.09
Fungi							
18:3ω6c	1.13	0.0006	1.53	0.0001	1.25	0.0002	0.76
18:1ω9c	7.26	0.0019	4.71	n.s.	4.77	n.s.	4.07
18:2ω6c	6.65	0.0024	4.12	n.s.	3.54	n.s.	3.15
Bacteria							
18:1ω7c	11.40	0.0001	4.51	n.s.	5.50	n.s.	3.30
15:0	0.66	0.0013	0.58	0.01	0.52	n.s.	0.50
*i*15:0	2.68	n.s.	3.39	0.08	3.29	n.s.	2.94
*a*15:0	1.92	n.s.	2.34	0.03	2.22	n.s.	1.97
*i*16:0	1.32	n.s.	1.77	0.04	1.72	0.07	1.47

† FAME, fatty acid methyl ester.
‡ Probability levels for the contrast comparison of the integrated crop–livestock and continuous cotton systems; n.s., not significant.
§ In the integrated crop–livestock system, the rotation was sampled under rye or wheat in November and under cotton or fallow in July.
¶ The values of the fatty acid abundance were averaged across 0–5, 5–10 and 10–15 cm depths and sampling time because depth and time were not significant ($P < 0.05$).

several microbial groups (Cavigelli et al., 1995; Zelles, 1999). Nonetheless, there are definite microbial markers such is the case of bacterial markers, which are rarely found in other cells (Cavigelli et al., 1995; Bossio et al., 1998; Zelles, 1999).

The differences in FAME profiles of perennial pasture compared with the rotation and continuous cotton demonstrated there were not only changes in microbial biomass, but also in the microbial community composition of this system, which affected the enzyme synthesis, and generated greater enzyme activities (Ndiaye et al., 2000; Schutter et al., 2001). The results appear to indicate that changes in FAME composition in perennial pasture were influenced by the higher abundance of protozoa (20:4ω6c), fungi (18:2ω6c, 18:3ω6c, and 18:1ω9c), and some bacteria (18:1ω7c and 15:0) in comparison with continuous cotton. However, the reason that other bacteria indicators remained unchanged in perennial pasture compared with continuous cotton, while there were changes in all the fungi indicators, could be related to its greater protozoa (20:4ω6c) levels, as they are involved on bacteria predation (Schutter et al., 2001).

Fatty acid methyl ester profiles showed no separation of the crop rotation and continuous cotton, however, there was higher abundance of some bacteria (*a*15:0, *i*16:0, *i*15:0, and 15:0) and fungi (18:3ω6c) indicators in the crop rotation when soil was sampled under rye or cotton compared with continuous cotton. In contrast, differences in FAME profiles and greater amounts of fungi (18:3ω6c and 18:2ω6c) in cover crop plots in comparison with winter fallow plots after 5 yr and in one grower field after 1 to 2 yr have been found (Schutter et al., 2001). Generally, higher fungi populations exist in systems that provide greater vegetative and litter cover, and lack of tillage (Frey et al., 1999; Schutter et al., 2001). The higher fungi (18:2ω6c) levels in the crop rotation, when soil was sampled under rye or cotton, are in agreement with the greater β-glucosaminidase ($r > 0.46$**) and arylsulfatase ($r > 0.58$***) activities found. Previous work has suggested the β-glucosaminidase activity is correlated to fungi biomass (Miller et al., 1998; Parham and Deng, 2000), and arylsulfatase activity is an indirect indicator of fungi (Bandick and Dick, 1999). Thus, it appears that fungi are a major component for the shift in the soil microbial community between a diverse crop rotation or pasture and continuous cotton.

The C_{mic}/N_{mic} ratio is often used to describe the structure and state of the microbial biomass (Moore et al., 2000), and thus, it allowed a comparison with FAME results. The C_{mic}/N_{mic} ratios >6 found indicate higher fungi with respect to bacteria populations in this soil (Jenkinson, 1976; Moore et al., 2000). This is in agreement with the higher abundance of some fungi indicators compared with the bacteria in this soil. However, the C_{mic}/N_{mic} ratio did not show the treatment effects observed by FAME analyses. This inconsistency may have occurred, as mentioned previously, due to the possible extraction of fatty acids from the soil matrix providing most likely a historical community structure assessment of the total fatty acids after 5 yr. However, some fungi indicators (18:2ω6c) were correlated with the microbial biomass C ($r > 0.77$***) in this soil. It is more likely that the C_{mic}/N_{mic} ratio was not a sensitive parameter to community structure changes as affected by management. Some researchers found that this ratio is relatively constant in arable soils and independent of soil moisture and temperature, N fertilization, development of the root system (Joergensen, 1995), and cropping systems (Moore et al., 2000). Semiarid soils have high pH and do not favor fungi. Despite this, the integrated crop–livestock system had higher fungal levels because fungi are generally favored by less soil disturbance (Frey et al., 1999).

Aggregate stability had similar management effects as microbiological parameters ($r > 0.55$**) and organic C ($r > 0.42$*) in perennial pasture (0–5 cm), which demonstrates the relationship between microorganisms and aggregation in soils. This follows previous work where winter cover crops had positive impacts on microbial biomass composition and aggregate distribution (Miller and Dick, 1995; Schutter et al., 2001). Aggregate formation by microorganisms can occur by their decomposition products or by mechanical binding of soil particles by fungal growth (Lynch and Bragg, 1985).

This study found clear and consistent differences in several soil properties between continuous cotton and perennial pasture. However, differences between continuous cotton and the crop rotation depended by which crop was sampled in the crop rotation and the soil parameter investigated.

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